be capable of sensing the interactions. Field-effect transistors (FET) have been widely used as biosensors, but are generally used with a large number of molecules to obtain a sufficient signal. The measurements taken with the FET-based biosensors are mostly ''on'' or ''off'' measurements, which are determined by the presence or absence of a reaction. A specific type of FET, the p-type metal oxide semiconductor FET (pMOSFET) appears to be a promising biosensor device. The pMOSFET contains holes in the channel, also known as the inversion layer, opposite in carrier type to the substrate. An atomic force microscope (AFM) has shown the ability to measure molecular interactions down to the single molecular level and to control the distance between a ligand and a receptor protein up to subatomic resolution. The integration of AFM and FET technologies has the potential to provide not only valuable information about these biomolecular interactions that has not been accomplished by other methods, but also a much more rapid drug screening technique. The ability to control single molecules will allow for the comprehensive study of biomolecular interactions at the single-molecular level. This presentation will show how the AFM and FET were integrated into one functioning biosensor. The efficiency at detecting single molecular binding and unbinding events will be demonstrated by probing the interactions between avidin-biotin complexes.

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Forces in T Cell Antigen Recognition

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There are strong indications that **mechanical forces are particularly relevant in immune recognition**. For the immune system, the enormous variety of antigenic ligands imposes a fundamental challenge to the discriminative power; the mechanism for discriminating between activating and non-activating ligands has remained enigmatic.

In a recent theoretical study we showed how forces alters the potency for receptor ligand discrimination by orders of magnitudes(1). For the T cell receptor, which specifically binds to peptides presented by MHC on an antigen-presenting cell, discrimination can be realized with kinetic proofreading, which fails when ligands have only marginal differences in their off-rates. We showed, however, that the **specificity of antigen-recognition can be massively improved** by putting the TCR-pMHC bond under load: while under no force the bond rupture probability decays exponentially with time, force-induced bond rupture leads to much narrower distributions.

Here, we present **cellular traction force microscopydata to measure forces involved during T cell activation.** Hydrogels were prepared with variable stiffness. Fluorescent beads carrying CD3 antibodies were immobilized onto the top layer of the hydrogel. Forces are read out by measuring the fluorescent bead movement throughout T cell attachment and activation. The bead movement was directly correlated to forces applied to the antibodies immobilized on the beads. Moreover, discrimination between lateral and transversal applied forces was possible by tracking the beads' positions in 3D.

1.Klotzsch, E., and G.J. Schütz. 2013. Improved Ligand Discrimination by Force-Induced Unbinding of the T Cell Receptor from Peptide-MHC. Biophysj. 104: 1670-1675.

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Novel Generation of Crosslinkers allows Single Molecule Force Spectroscopy on Oligomeric Receptors

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Single molecule force spectroscopy allows for measurement of the unbinding forces between a tip-tethered ligand and a cognate receptor molecule which is immobilized on the sample surface. Conventionally, the atomic force microscope (AFM) tip is aminofunctionalized and the ligand is coupled via a linear heterobifunctional poly(ethylene glycol) (PEG) linker. This approach restricts the use of force microscopy to the investigation of single ligand-receptor pairs. For extension of the technique to the analysis of dimeric or oligomeric receptors, new crosslinkers carrying two or four terminal coupling groups for ligand-attachment were synthesized.

The syntheses are based on tri- and pentavalent core units. Heterobivalent PEGs serve as elongations to create the optimal ligand-to-ligand distance for each ligand-receptor system. In case of the bivalent linker, lysine was chosen as branching element and the biotin-streptavidin couple served as test system. The probability density function (histogram) of the unbinding forces showed two peaks, reflecting mono- and bivalent binding of the tip-bound bis-biotin linker to one support-bound streptavidin molecule. The tetravalent linker was prepared from three copies of a symmetric trifunctional subunit formed from lysine which carries a β-alanine on its α-amino group. In a multistep reaction, a 2*2 ''fork'' with four terminal amino groups was synthesized. Fortunately it was possible to elongate each prong of the 2*2 fork with a PEG chain of the same length, using HATU and HOAt for in-situ coupling of amine and carboxyl groups. Terminal azido-groups provide for coupling of alkyne-functionalized ligands directly on the tip. In conclusion, branched crosslinkers permit single molecule force spectroscopy on oligomeric receptors; their modular structure can easily be adapted for different ligand-receptor-system in terms of coupling functions and ligand-to-ligand distance.

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Design and Optimization of a High Force Neodymium Iron Boron based Magnetic Tweezers Device using Finite Element Analysis

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We present the design and characterization of a high force magnetic tweezers device that can apply controlled forces to magnetic beads embedded into soft materials or biological systems, while visualizing the resultant material deformation with microscopy. Using finite element analysis (FEA), we determined the effect of the geometry of the NdFeB magnet array, as well as the geometry of iron yokes designed to focus the magnetic fields. Sixteen shape parameters including the magnet size, positioning and yoke curvature were defined and modeled using open-source magnetic FEA software. Parameter sweeps were performed using custom-written Matlab code. Geometries were optimized for the magnitude of the magnetic field gradient and the length scale over which the magnetic force operated. Once an optimal design was identified, the yoke was fabricated in-house and the FEA validated by mapping the device's magnetic field. To demonstrate the usefulness of this approach, we produced a magnetic tweezers device designed for use with optical microscopes available in a core imaging facility. The application demanded device portability and the ability to interface with a number of microscopes, thus imposing significant size restrictions on the magnets used. Iterative FEA delivered an optimal magnet-yoke geometry, which could be mounted to a carriage that advances or retracts on command, giving the operator fine control over the applied force. Such automation allows for rapid force switching, and also allows the effects of long periods of cyclical loading to be determined. In future work, such an FEA approach could easily be adapted to a range of design goals/restrictions to create an efficient means of testing possible magnet configurations, while streamlining the design and construction of specialized instrumentation for force-sensitive microscopy.

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smAP: Manipulating DNA by Ultrasound - Single-Molecules Go Acoustic

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The ability to study individual biomolecules in vitro has greatly expanded our knowledge of biological systems. Existing single-molecule techniques, such as optical and magnetic tweezers or atomic force microscopy, allow manipulation of individual biomolecules like DNA. They suffer from either being technically challenging or they offer low experimental throughput. We invented a novel lab-on-a-chip method to exert controllable forces on multiple DNA molecules simultaneously using ultrasound: single molecule Acoustical Pushing (smAP). smAP consists of a resonator integrated into a micro-fabricated fluidic chip. An acoustical pressure gradient is created homogeneously throughout the sample enabling to exert forces on DNA-tethered beads. By changing the amplitude of the driving voltage the pressure gradient can be altered, allowing sensitive control of the force applied to the DNA molecules.

This approach makes it possible to apply forces up to hundreds of picoNewtons homogeneously over an area of several millimeter squared, allowing multiplexing to an unprecedented level. We validate this novel single-molecule method by recording force-distance curves of DNA molecules, both double- and single-stranded, in the presence of DNA-binding proteins. The simplicity and low cost makes smAP a widely accessible tool for biophysicists.

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An AFM Force Pulling Study of Riboflavin Receptor Targeting Nanoparticles

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Riboflavin ligands present an alternative pathway for targeted drug delivery as riboflavin receptors are over-expressed in breast and prostate cancer cells. We have examined a riboflavin-conjugated PAMAM dendrimer (generation 5) for